Adoption of a "mini-prep" DNA extraction method for RAPD marker analysis in Common Bean (Phaseolus vulgaris L.).

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Several forms of biochemical or molecular markers (protein, isozyme, RFLP, and RAPD markers) have been used for genome analysis and marker-assisted selection in dry beans (Phaseolus vulgaris. L). In our breeding program, we have chosen the RAPD marker primarily due to the simplicity of analysis when compared to RFLP technology. However, the efficient preparation of DNA samples for either molecular analysis is considered by many a meaningful objective.

We have adopted a simple and quick DNA extraction method that resulted from the combination of two other methods. Miklas et al.(1993) developed a large scale DNA extraction process using 5 g of fresh tissue collected from bean plants about 10% bloom. In addition to the large amount of tissue required with this method, lyophilization and grinding are necessary before the extraction process is initiated. Even though this method yields a large quantity of DNA, it imposes limitations of time, labor, and cost. Combining the large scale DNA extraction method, and the "mini-prep" protocol of Edwards et al.(1991), we have developed a DNA extraction method which works well with beans. Using this process 50 DNA extractions can be completed within four hours by a single worker.

The major modifications to Edwards' protocol were the choice of extraction buffer, use of chloroform-isoamyl alcohol(24:1), and addition of RNAse. We found that using the CTAB buffer instead of Tris buffer for DNA extraction, resulted in elimination of most of the plant debris. Accumulation of plant debris because of DNA extraction may inhibit PCR (Innis and Gelfand, 1990). The addition of RNAse makes samples more suitable for PCR analysis by reducing the chances of RNA amplification.

Tissue for DNA extraction can be collected from any young primary(4 to 7 days post-emergence) or trifoliate(newly expanded) leaf from greenhouse or field grown plants (use the youngest possible tissue). Good results are also obtained using about 100 μl of lyophilized ground tissue. One or two discs of leaf tissue are cut by clipping the leaves with a 1.5 ml Eppendorf tube. Each tube is then kept on ice until all samples are collected. Samples can be processed immediately, or they may be stored in the freezer for several days without affecting DNA yield.

Extraction is initiated by maceration of leaf tissue with a mini-pestle (Kontes # K7-4952000 from Fisher) until all tissue is disrupted. Following this initial maceration procedure, 100 μl of hot (65 °C) CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, 1% ß-mercaptoethanol) are added to each tube. After the sample is well homogenized, an additional 300 μl is added to each tube. Once the sample is completely ground, the tubes are allowed to incubate for 20 min at 65 °C. Following incubation, 400 μl of chloroform-isoamyl alcohol(24:1) are added to each tube, and mixed on a shaker for 15 min. The extracts are centrifuged at 13,000 rpm for five min, and the supernatant transferred to a new Eppendorf tube containing 400 μl of isopropanol. Samples are mixed well and left at room temperature for five min. Following centrifugation at 13,000 rpm for five min, the supernatant is discarded and the tubes are inverted for five min to dry the pellet. The pellet is resuspended in 100 μl of $TE_{0.1}$ buffer (Tris- EDTA 0.01mM pH 8.0), and 4 μl of RNAse (10 mg/ml) is added to each tube. Samples are left at room temperature for 15 min before precipitation with cold 100% ethanol for 15 min. Samples are centrifuged and the supernatant discarded. The pellet is resuspended in 100 μl of $TE_{0.1}$ buffer.

Bean DNA extractions from more than 1,500 samples have yielded an average of 78 ng/μl of DNA, in a final volume of 100 μl of TE buffer. These results indicate the usefulness of this method in terms of yield, time expended during the extraction process, amount of reagents (less than 1 ml for all reagents per sample), labor, and amount of tissue required. We have observed a high degree of variability in DNA yield among samples with this extraction method. The causes of such variation could be differences in genotype, leaf thickness, number of cells per unit area, or environmental conditions present in the field or greenhouse. Although variability in DNA yield occurs, over 99% of the samples extracted have yielded enough DNA to conduct numerous PCR analyses. We have also verified that this extraction method yields stable DNA. The DNA extracted in this way has been used to screen for seven RAPD markers using eleven segregating populations, in addition to surveying a collection of Mesoamerican and Andean bean germplasm for RAPD variability.

Another major advantage of this method is that it allows the screening of individual plants without sacrificing too much tissue. Sampled plants can be used for further sampling, disease screening, crossing, and seed production. The procedure does not adversely affect maturity or seed production even when samples are collected from primary leaves. Sampling can also be done during any developmental stage since the smallest leaf on a plant usually provides enough tissue for DNA extraction. The simplicity of this method enables the training of student workers or any inexperienced laboratory personnel.

We consider this method to be very useful for our purposes. This protocol has reduced considerably the expenses and time invested per processed sample, allowing us to obtain more results in a shorter time period.

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